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NADH:ubiquinone oxidoreductase (Complex I, CI) is one of the major sources of reactive oxygen species (ROS) in mitochondria. ROS are critically involved in several degenerative diseases like Leigh syndrome, Leber's hereditary optic neuropathy or Parkinson's disease. CI-dependent ROS formation occurs at the NADH-site and is driven by the fully reduced flavin mononucleotide (FMN) rather than the semireduced FMN radical. The importance of several conserved amino acids in vicinity to FMN has been shown by structural analysis. Glu^{95}, Glu^{97} and Tyr^{180} in NuoF from *Aquifex aeolicus* (human orthologue NDUFV1) are suggested to be involved in electron transfer from NADH to FMN. Furthermore, Tyr^{180} is of pathophysiological relevance indicated by a mutation in a CI-deficient patient (human orthologue Tyr^{204}).

To investigate the contribution of these amino acids on ROS formation we used a surrogate model of CI. We coexpressed NuoE and NuoF from *A. aeolicus* in *Escherichia coli*, which results in soluble NuoEF protein, containing FMN and the Fe–S-clusters N3 and N1a. Site-directed mutagenesis in NuoF at the positions Glu^{95}, Glu^{97} and Tyr^{180} was used to generate mutant NuoEF. The (mutant) proteins were investigated by steady state kinetics and redoxtitrations, to assess the impact of the respective amino acids on ROS formation. Wild type NuoEF protein generates ROS in a $FMNH_2$-dependent manner at rates of 110 nmol/min*mg. FMN potential (E^0) is −0.320 V (at pH 7.5) which is comparable to data obtained from CI in *E. coli*. Mutations in NuoF critically affect ROS production, e.g. Y180A showed 4-fold higher ROS production, contrasted by Y180L where ROS production was reduced to 40%. Interestingly, these mutant proteins show a different behavior in redoxtitrations pointing to a mechanistic change of ROS production by CI.

These findings indicate that small changes in the structural environment of the FMN substantially affect the potential or stability of different FMN species which affect ROS formation and might have implications for diseases associated with CI-dysfunction.

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6P2

The accession gate for ubiquinone of complex I from *Yarrowia lipolytica*

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Mitochondrial NADH:ubiquinone oxidoreductase (complex I) is an L-shaped membrane protein that has a central function in oxidative phosphorylation. The overall architecture of complex I was determined using X-ray crystallography, however the mechanism of redox-driven proton-pumping remains to be elucidated. Electrons are transferred from NADH via a chain of Fe–S clusters to reduce ubiquinone bound in a deep binding pocket that is comprised of the 49-kDa and PSST subunits of complex I. Iron–sulfur cluster N2, the immediate electron donor for ubiquinone, resides about 30 Å above the membrane domain. The binding of the ubiquinone head group is mainly stabilized by hydrogen bond interactions to the conserved Y144 of subunit 49-kDa. To investigate the pathway of the hydrophobic tail, five chemically modified ubiquinone derivatives were applied as substrates in enzyme kinetics measured with mitochondrial membranes of complex I mutants. We concluded from these extended structure/function analyses that the tail of ubiquinone enters through a narrow hydrophobic path at the interface between the 49-kDa and PSST subunits. Furthermore, we identified several conserved methionines that seem to form a structural and functional hydrophobic gate to the active site reminiscent to the M-domains of the signal recognition particle of the endoplasmic reticulum. To gain further insight into the architecture of the ubiquinone binding domain, five amino acids within the binding pocket of the 49-kDa subunit of *Yarrowia lipolytica* were changed by site-directed mutagenesis to match the amino acid pattern of bovine complex I in this region. We could show that residue S192 is responsible for the decreased sensitivity of yeast complex I towards the inhibitor rotenone.

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6P3

Mapping of A/D conformational changes of mitochondrial complex I

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Complex I or NADH:ubiquinone oxidreductase is the first and largest enzyme of the respiratory chain. Mammalian mitochondrial complex I is composed of at least 45 subunits with a molecular weight of 980 kDa. Despite recent progress in structural studies, many aspects of the regulation of the enzyme are still not clearly understood. Mammalian enzyme can exist in catalytically active (A) form and de-active, dormant (D) form. When the turnover of the enzyme is limited the A-form undergoes reversible de-activation at physiological temperatures. Until now, the exposure of Cys 39 of the ND3 subunit after de-activation is the only conformational difference observed between the A and the D-form, accounting for the sensitivity of the D-form to SH-reagents [1].

Using two homogenous preparations of bovine heart submitochondrial particles containing either the A or the D-form of complex I, we observed no difference in the profile of respiratory chain enzymes shown by Blue Native PAGE [2]. Moreover, Cys 39 is accessible for SH-reagents in the D-form of the enzyme even when complex I is a part of respiratory chain supercomplexes. To characterise the relative location of Cys 39 of the ND3 subunit we used internal protease bromoacetamidobenzyl-EDTA (Fe-BABE)-SH-reagent for differential